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10/089,040	04/16/2002	Yoshio Umezawa	2002-0426A	9569

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WENDEROTH, LIND & PONACK, L.L.P.  
2033 K STREET N. W.  
SUITE 800  
WASHINGTON, DC 20006-1021

EXAMINER

MOORE, WILLIAM W

ART UNIT	PAPER NUMBER
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1652

DATE MAILED: 06/02/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

# Office Action Summary

Application No.

10/089,040

Applicant(s)

UMEZAWA ET AL.

Examiner

William W. Moore

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

## Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

## Status

- 1) ☒ Responsive to communication(s) filed on 11 March 2005.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

## Disposition of Claims

- 4) ☒ Claim(s) 1,3 and 6-11 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1,3 and 6-11 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

## Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

## Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

## Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)  
Paper No(s)/Mail Date 20020326.
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date. \_\_\_\_\_.
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: \_\_\_\_\_.

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## DETAILED ACTION

*Response to Amendment*

Applicant's Amendments to the specification and claims filed 11 March 2005 has been entered, canceling claims 2, 4 and 5, amending claims 1, 3, 6, 8, 10 and 11, and clarifying somewhat the disclosures at pages 11 and 17 of the specification. While one amendment to page 17 of the specification is inadequate for clarification of the intent of the disclosure, it is agreed that the amendments to pages 11 and 17 of the specification add no new matter: The term "self-excision" at page 10 of the specification supports the amendatory term "autocatalytic" and page 9 of the specification explains that intein splicing, or ligation, accompanies intein excision. Figure 2 depicts three locations in the amino acid sequence of the prior art enhanced green fluorescent protein [EGFP] where the "m125" and "m126" modifications introduce, respectively, double and triple amino substitutions. New matter in the amendment to page 18 of the specification is indicated below and claims 1, 3 and 6-11 are pending and examined herein.

*New Matter Objection*

The amendment adding a sentence at page 18 of the specification filed 11 March 2005 is objected to under 35 U.S.C. § 132(a) because the sentence introduces new matter into the disclosure. 35 U.S.C. § 132(a) states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: 1. No fusion polypeptide is encoded by a cDNA. 2. There is no indication in the specification that any fusion polypeptide was prepared linking the amino terminus of the *S. cerevisiae* VDE intein, which is a cysteine according to page 16 of the specification, to the amino-proximal 128 amino acids of either a native or a mutant EGFP, and also linking amino acids 129 through 238 of an EGFP to the carboxyl terminus of the VDE intein, an asparagine according to page 16

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of the specification. Figure 2 depicts no division of an EGFP amino acid sequence that partitions the sequence of positions 1 through 128 to one probe and the sequence of positions 129 through 238 to another probe and Figure 4 does not depict such division. The text and the other figures of specification do not otherwise indicate how a mutant EGFP is to be divided by an intein, or by intein components, to prepare probes "a" and "b". Applicant is required to cancel the new matter in the reply to this Office Action.

#### *Objection to the Specification*

The disclosure remains objected to because of numerous informalities: 35 U.S.C. § 112, first paragraph, requires the specification to be written in "full, clear, concise, and exact terms." The amendments to lines 12-16 at page 17 of the specification do not correct any of the grammatical informalities in the two sentences and the second sentence remains unclear and inexact because the phrase "mutation **at** m125" is illogical where the term m125 indicates two specific mutations in a specific EGFP amino acid sequence, one of which occurs **at** position 125 of the native EGFP sequence. The other occurs, see Figure 2, at position 129 of the native EGFP sequence. The Office communication mailed 6 October 2004 pointed to the most perplexing examples of unclear, inexact, or verbose terms used in the specification, but several inexact or unclear terms appear at every page of the specification. The problems are diverse and include frequent omissions of the definite article "the", where it should be employed, the lack of spacing between words, improper proper verb number and tense, and unclear characterizations. One instance of a combination of these various kinds of informalities is the short paragraph at the close of page 17 of the specification: (i) the description of two mutations in the first sentence of the paragraph requires the use of the plural noun "mutations" and the use of the plural form of the verb "to be", (ii) the definite article "the" is required for three terms in the first sentence but is not present, and (iii) it is not clear

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how the mutant, which is an entire polypeptide, becomes a "labeled protein site" where nothing is added to the EGFP to label it. Instead, the mutant EGFP reconstituted by ligation mediated by intein components of a claimed probe is more properly described as an indicator, not a label, of protein-protein interaction. The specification is replete with terms that are not clear, concise and exact, and correction or clarification of the numerous grammatical and logical inconsistencies throughout the specification is required in response to this communication.

This application claims benefit to a provisional application No. 2000-224939, filed on 26 July 2000, in a language other than English. Applications that claim the benefit of a provisional application filed in a non-English language must include an English translation of the non-English language provisional application and such a translation was indeed provided at the time this application was filed under 35 U.S.C. § 371. That translation might be inadequate in describing the nature of the invention. If Applicant considers that the informalities in the specification stem from an inadequate translation, and particularly if Applicant considers that a proper translation can assist in resolving the problems indicated in the following rejections of the claims, Applicant is invited to submit a translation that is certified to be accurate in response to this communication.

#### *Claim Objection*

Claims 1 and 11 are objected to because of the following informalities: Use of parentheses in the terminal clause of claim 1 is inappropriate. The term "eucaryotic" in claim 11 does not represent the standard spelling, "eukaryotic". Use in claim 11 of the phrase "coexist in the cell" is superfluous because expression of polypeptides in a host cell that each comprise one of two target proteins for analysis already assures that they are present in an eukaryotic host cell for analysis of emissions from the portions of the indicator protein should the target proteins interact. Appropriate correction is required.

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*Claim Rejections - 35 USC § 112*

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 3, 6-8, 10 and 11 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the preparation of a probe of claims 1, 3, 8 and 9 comprising an indicator which is a firefly luciferase divided at the amino acid positions 437 and 438 into N-terminal and C-terminal portions, and the practice of methods of claims 10 and 11 for the analysis of protein-protein interaction utilizing probes that comprise such N-terminal and C-terminal portions of a firefly luciferase, does not reasonably provide enablement for preparation of probes of claims 8 and 9 comprising a generic luminescent enzyme or probes of claims 6 and 7 comprising an indicator which is a generic fluorescent protein, such as a native EGFP, or the practice of methods utilizing such probes. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

This is a new ground of rejection. It is agreed that the prior art of record herein teaches how to delete non-essential portions of inteins and further teaches how to divide, e.g., the *S. cerevisiae* VMA and *Synechocystis* DnaE inteins into the separate functional amino-terminal and carboxyl-terminal components described by claim 1 that can mediate the ligation of polypeptides fused to either of the intein components. Figure 4 of the specification demonstrates such division of the VDE intein. Examples 5 and 6 of the specification disclose one solution, pertinent to claim 9, of the special technical problem set forth at pages 14-16 of the specification: probes (a) and (b) are prepared by specific division of the amino acid sequence of the luminescent enzyme luciferase and these regions, divided into amino-terminal and carboxyl-terminal portions of luciferase shown in Figure 7, are fused with intein components so that the activity of the enzyme will be recovered upon ligation of the two luciferase portions. In the case of claims 1, 3, 8 and 9, amendments indicating the specific division of the firefly luciferase – between position 437 and position 438 – will overcome this rejection. Absent such

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amendment, claims 1, 3, 8 and 9 contemplate the preparation of probes “a” and “b” by dividing a generic luminescent enzyme, or a generic luciferase, into regions fused to intein components in order to retain enzymatic activity after a ligation mediated by intein splicing. Yet neither the prior art of record herein nor the instant specification, taken together, teach how one skilled in the art how to identify the locations of suitable positions in amino acid sequences of generic luminescent enzymes, where a useful division of the integral amino acid sequence of either can be made to ensure that probes of claims 1, 3, 8, and 9 will function upon ligation of their divided indicator components mediated by splicing of intein components of the probes or permit the practice of the methods for analysis of protein-protein interaction of claims 10 and 11 that require such probes.

Similarly, neither the prior art of record herein nor the instant specification, taken together, teach how one skilled in the art how to identify locations of suitable positions in amino acid sequences of generic fluorescent proteins, or suitable positions in a generic green fluorescent protein, where a useful division of the integral amino acid sequence of either can be made to ensure that probes of claims 1, 3, 8, and 9 will function upon ligation of their divided indicator components mediated by splicing of intein components of the probes or permit the practice of methods for analysis of protein-protein interaction of claims 10 and 11 with such probes. Instead, the specification teaches that a specific mutant EGFP – the m125 mutant having the dual substitutions E125I and I129C in the native EGFP amino acid sequence – must be prepared for division into N-terminal and C-terminal indicator protein segments and teaches, at page 18, only that the amino terminal portion of this divided, mutant, fluorescent protein must include “125 amino acids from the N-termin[us]”. There is no clear teaching of any other particular locus for dividing the mutant EGFP amino acid sequence in order to solve the special technical

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problem and allow the preparation of probes of claims 6 and 7. Where the specification itself fails to provide the critical teaching of where to divide the amino acid sequence of the disclosed mutant EGFP to ensure the recovery of fluorescence upon ligation of the separated regions of the EGFP it is not clear what amendment might be proposed to overcome this rejection as it is applied to claim 1, 3, 6 and 7.

It is well settled that 35 U.S.C. § 112, first paragraph, requires that a disclosure be sufficiently enabling to allow one of skill in the art to practice the invention as claimed without undue experimentation and that unpredictability in an attempt to practice a claimed invention is a significant factor supporting a rejection under 35 U.S.C. §112, first paragraph, for non-enablement. See, *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), discussing eight factors relevant to an analysis of enablement. The standard set by the CCPA is not to "make and screen" any and all possible amino acid sequence alterations because a reasonable correlation must exist between the scope asserted in the claimed subject matter and the scope of guidance the specification provides. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 25 (CCPA 1970). The Federal Circuit approved this standard set by the CCPA in *Genentech, Inc. v. Novo-Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). Applying the factors for analysis discussed in *Wands*, *supra*, to Applicant's disclosure, it is apparent that:

- a) the specification lacks adequate, specific, guidance for determining how to divide amino acid sequences of generic fluorescent proteins, even the disclosed mutant EGFP, or amino acid sequences of generic luminescent enzymes to assure the recovery of fluorescence, or enzymatic activity, upon ligation,
- b) the specification lacks working examples wherein a process of determining how to divide amino acid sequences of generic fluorescent proteins or generic luminescent enzymes is taught that can assure the recovery of, respectively, fluorescence or enzymatic activity,
- c) in view of the prior art publications of record herein, the state of the art and level of skill in the art do not support such alteration, and,



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d) unpredictability exists in the art which does not teach the division of amino acid sequences of generic fluorescent proteins, even the disclosed mutant EGFP, or the division of amino acid sequences of generic luminescent enzymes so as to assure recovery of, respectively, fluorescence or enzymatic activity upon ligation of the divided amino acid sequence.

Thus the present specification fails to enable the preparation of the claimed probes, and the practice of claimed methods requiring the probes, even if taken in combination with the teachings available in the prior art of record.

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3, 6-8, 10 and 11 are rejected, in part for reasons of record, under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection of record of claims 1, 3, 6-8, 10 and 11 for indefinite description is maintained because the claims still fail to describe the structural relationship, i.e., the orientation, of the intein and indicator protein components of the probes "a" and "b". Although the claim amendments filed 7 March 2005 improve the recitations of the pending claims, the amended claim 1 still fails to indicate that the intein portions of a probe are fused directly to an indicator protein region of a probe and the order in which these components are fused in preparing both of the probes "a" and "b". The public and the artisan seeking to ascertain the metes and bounds of the intended subject matter cannot determine the positions of components in probes of claims 1 and 6-8, determine where linkers of claim 3 occur, or determine where and how proteins in claims 10 and 11 are "linked" to probe "a" or probe "b" to allow analysis of protein-protein interaction. Claims 3 and 6-11 are rejected together with claim 1 because they do not resolve its indefinite description. Amending claims 1, 3, and 10 to describe structural relationships of indicator protein components, intein components, linker components, and target

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protein components, i.e., the order in which they are consecutively fused to one another, see, e.g., Figures 4 and 5, will overcome this aspect of the rejection.

Claims 1, 6 and 8 are independently indefinite reciting "labeled protein" to describe components of probes "a" and "b". The specification discusses two genera of proteins, fluorescent proteins and luminescent enzymes, that function as radiant indicators of protein-protein interaction upon intein-mediated ligation of previously divided regions but does not disclose that any radiant indicator protein is "labeled", whether in integral form or when divided into probes "a" or "b". The term is probably an artifact of translation and is not functionally descriptive. Claims 3, 7, 9, 10 and 11 are included in this rejection because they depend from claims 1, 6 and 8 but do not resolve the indefinite description of the claims from which they depend. This aspect of the rejection may be overcome by claim amendments that replace the term "labeled" with a functionally descriptive term.

Claim 10 is independently indefinite because the method it describes is incomplete where each protein that is a potential partner in an analysis of protein-protein interaction must first be fused to a component preexisting in one or the other of the probes "a" and "b". As the specification discloses, this preparation step is a prerequisite for a further "contacting" step. Claim 10 is also indefinite where it recites term "linked" because the specification does not disclose a linking of either target protein to probes "a" or "b" and instead discloses that one target protein is fused to the carboxyl terminus of one probe while the other target protein is fused to the carboxyl terminus of the other probe, see Figure 5. Claim 11 is included in this rejection of claim 10 because it fails to resolve the ambiguities of claim 10.

Claim 11 is independently indefinite in reciting that a "polynucleotide" "expresses" a protein. Instead, as depicted in the final vector construct of Figure 7, a polynucleotide used for the practice of a method for analysis in eukaryotic cells actually encodes two

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separate fusion polypeptides and the polynucleotides does not express itself: it is the host cell that expresses both fusion polypeptides encoded by the polynucleotide.

*Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 6, 10 and 11 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Remy et al., 1999, and Wu et al., 1998a, in view of Wu et al. 1998b, cited in the specification and made of record herewith.

Claims 1, 6, 10 and 11 do not require that a probe serving as an indicator of protein-protein interaction be a fluorescent protein that is not an enzyme or that a claimed method utilize such a probe. Remy et al. teach the preparation of a bipartite, enzymatic, probe for the detection and quantitation of protein-protein interaction wherein fragments of an enzyme are each separately, recombinantly, fused to target proteins having a presumed interaction that can be detected, thereby forming fusion polypeptides that are encoded by separate coding regions comprising a split operon within a polynucleotide suitable for common expression of the fusion polypeptides, hence their coexistence, in an eukaryotic host cell. See pages 5394, 5395, 5398 and 5399. Remy et al. further teach that the different regions of the divided enzyme fused separately to each target protein come together to reconstitute the enzyme's activity if the target proteins present in the fusion polypeptides interact, and that this permits detection of the interaction of the target proteins by complementing a lack of the enzyme's activity in the host cells that permits cell survival as well as a method for the quantitation of the extent of binding by fluorescence microscopy spectroscopy wherein a change in fluorescence intensity resulting from protein-protein is measured where one substrate bound by their selected

indicator enzyme provides fluorescence when bound by the reconstituted enzyme. See pages 5395-5398 and Figures 1-5. Remy et al. do not teach that a split intein should be partitioned into the separate fusion polypeptides between the target protein portion and the separate enzyme portions but do teach, page 5399, that "the design of this system begins with the dissection of a small monomeric enzyme of known structure" and that "because the enzyme is monomeric and results from association-folding of two fragments . . . an observed response is caused by a binary protein-protein interaction.

Wu et al., 1998a, teach that the naturally-occurring *Synechocystis* sp. DnaE intein has, see Figures 1, 2B, 3A and 3B, an amino terminal portion and a carboxyl terminal portion that are each separately fused to separate portions of the DnaE protein in separate genes widely separated in the cyanobacterium's genome, that this protein is a component of a DNA polymerase essential for DNA replication in the cyanobacterium's cells, and that the intein's amino terminal and carboxyl terminal portions will trans-splice the enzyme component upon recombinant expression from a polynucleotide comprising a split operon in a transformed host cell. Wu et al., 1998b, teach that trans-splicing mediated by amino terminal and carboxyl terminal portions of another cyanobacterial intein, the DnaB intein, that has been split into the two portions will produce a covalent bond joining polypeptides other than the native DnaB helicase when the heterologous proteins are fused to, respectively, the amino terminus of the amino terminal intein portion and the carboxyl terminus of the carboxyl terminal intein portion and the separate fusion polypeptides are recombinantly expressed from a polynucleotide comprising a split operon in a transformed host cell. See Figures 2 and 3.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to prepare a probe for analyzing protein-protein interaction between two proteins by preparing polynucleotide encoding two fusion polypeptides, each comprising

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a separate fragment of a divided protein that is capable of fluorescence wherein one fragment is fused to an amino terminal portion or a split intein and the other fragment is fused to a carboxyl terminal portion of a split intein and wherein each portion of the split intein is in turn fused to either of two proteins the interaction of which is to be analyzed upon expression of the two fusion polypeptides in an eukaryotic host cell. This is because Remy et al. teach that a bipartite, fluorescent indicator protein serving as a probe for the detection and quantitation of protein-protein interaction can be split into fragments that are each separately, recombinantly, fused to target proteins having a presumed interaction to be analyzed and that the separate portions of an indicator protein that is an enzyme will provide a fluorescent signal when brought into close proximity by interaction of the target proteins to reconstitute its activity and because Wu et al., 1998a, teach that an enzyme split into two separate portions can be ligated to reconstitute activity by a split DnaE intein, the amino terminal and carboxyl terminal portions of which are fused to either portion of the split enzyme, wherein the split DnaE intein will splice the separate portions into a free, integral, enzyme. Such an artisan would have had a reasonable expectation of success in combining either portion of the split enzyme and either of the target proteins to either portion of the split DnaE intein because Wu et al., 1998b, demonstrate that another split intein will splice heterologous fusion partners into a free, integral, polypeptide. Such an artisan would also have appreciated that intein-mediated splicing to reconstitute the fluorescence of an indicator protein would be more effective in providing a fluorescent signal for analysis of protein-protein interaction because the split intein portions themselves have adequate affinity for each other, see Figure 4 of Wu et al., 1998b, to assure proper association of an indicator protein and because a spliced, free, indicator protein would persist in the cell in which it's component portions had been expressed in separate fusion polypeptides.

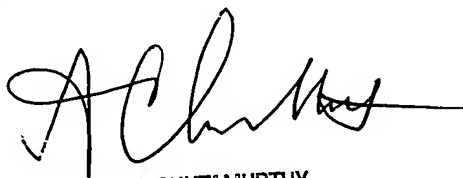
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*Conclusion*

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is now 571.272.0933. The examiner can normally be reached between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can now be reached at 571.272.0928. The fax phone number for all communications for the organization where this application or proceeding is assigned is now 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is now 571.272.1600.

William W. Moore  
25 May 2004



PONNATHAPU ACHUTAMURTHY  
SUPERVISORY PATENT EXAMINER  
TECHNOLOGY CENTER 1600